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Skeletal Muscle Tissue Oxygen Pressure Distribution During Early Reperfusion After Prolonged Ischaemia

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Objectives: *the aim of this study was to investigate the skeletal muscle tissue oxygen pressure (P_tO_2) distributions during early reperfusion (10–45 min) after prolonged ischaemia in a rat animal model.*
Material and methods: *skeletal muscle ischaemia was induced in anaesthetised rats by applying a tourniquet on the left thigh for 3 h (group I) or 4 h (group II), and tissue oxygen pressure measurements were made after 10–45 min of reperfusion. Assessment of P_tO_2 was made by a multiwire Clark-type oxygen microelectrode, placed on the surface of the left tibialis anterior muscle.*
Results: *during reperfusion a similar P_tO_2 pattern was evaluated after both 3 and 4 h of total ischaemia, where the sum P_tO_2 distributions were shifted to the left associated with low tissue oxygen pressure values. After 10 min of reperfusion the median P_tO_2 was 0.28 kPa and 0.18 kPa, in groups I and II, respectively; after 45 min of reperfusion 0.61 kPa and 0.60 kPa, respectively. The median P_tO_2 in the non-ischaemic muscle in groups I and II were 2.19 and 2.17 Pa.*
Conclusion: *the results show that local skeletal muscle oxygenation is severely impaired during the initial 45 min of reperfusion after both 3 and 4 h of total muscle ischaemia with a slow-reflow phenomenon generally present, despite pronounced needs.*

Key Words: ischaemia, reperfusion injury, skeletal muscle, tissue oxygenation.

Introduction

Restoration of tissue perfusion and oxygenation after a period of impaired circulation is an important clinical objective in many medical conditions. Only a few minutes of hypoxia in the brain can be deleterious, whereas other tissues are more tolerant. Heart muscle can sustain some 45–60 min of ischaemia before irreversible injuries develop. The skeletal muscle is more tolerant to ischaemia and can survive up to 3–4 h of total ischaemia.

The development of both macro- and microsurgical techniques for revascularisation, bypass surgery and replantation has led to a demand for the assessment of the possible safe extension of the duration of total ischaemia in various tissues, before reperfusion can be accomplished clinically. Despite the pronounced anoxic metabolic consequences in the ischaemic tissue, it has been known for a long time that the initial reperfusion flow rates following prolonged ischaemic periods are very low; the slow- or no-reflow phenomenon.[1] The mechanism behind this post-ischaemic reperfusion impairment and the potential prolongation of damaging hypoxia is not fully understood, but several mechanisms have been proposed, such as post-ischaemic endothelial swelling,[1] increased post-ischemic tissue oedema,[2] increased expression of tethering and adhesion molecules on the surfaces of the microvascular endothelium causing increased rolling and sequestering of WBC.[3] Earlier studies have demonstrated an impaired microcirculation in skeletal muscle during reperfusion after prolonged ischaemia,[4–7] but the effect of ischaemia on muscle tissue oxygenation is less settled.

The present study was undertaken in order to study the skeletal muscle tissue oxygen pressure (P_tO_2) distribution during the initial 10–45 min of reperfusion after 3 and 4 h of total ischaemia. The skeletal muscle tissue oxygen pressure, P_tO_2, was measured with a Clark-type surface multiwire microelectrode and evaluated by means of P_tO_2 histograms.[8–10]

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Materials and Methods

Surgical preparation

The experiments were carried out on a total of 12 Sprague–Dawley rats, weighing 200–300 g. They were anaesthetised intraperitoneally with a mixture of 50 mg/kg ketamine (Ketalar®) and 5 mg/kg xylazine (Rompun®). The animals were spontaneously breathing and kept under continuous surgical anaesthesia throughout the experiment by additional intraperitoneal doses of the anaesthetic mixture, as necessary. The animals were randomised into two groups: group I (six animals) had one leg exposed to a total leg ischaemia for 3 h, using the tibialis anterior muscle as the study object, and group II (six animals) had one leg exposed to 4 h of ischaemia. Leg ischaemia was accomplished using a rubber tourniquet placed proximally around the upper, left thigh. This model has been validated and used repeatedly in our laboratory for a number of studies of total ischaemia of skeletal muscle.[1–6] Reperfusion was accomplished by cutting the rubber band. After release of the tourniquet the skin and fascia over the left tibialis anterior muscle surface was surgically opened and a lucite electrode holder holding the electrode was positioned with utmost care, so that the multiwire electrode tip came in contact with the fluid immediately over the muscle surface, but not causing any pressure on the muscle surface. It was possible to turn the electrode around in its holder, without affecting or touching the underlying muscle surface. The skin and fascia were thereafter closed around the electrode holder by suturing to prevent ambient air from affecting muscle surface oxygenation.[11] The normally perfused, contralateral tibialis anterior muscle in each animal was used to sample normal, control values for P_tO_2.

The animal experiments were approved of by the local ethical committee. Animal care complied with the Principles of Laboratory Animal Care, as formulated by the Swedish Medical Research Council and with the guide for the care and use of laboratory animals in NIH Publication No. 86–23, revised in 1985.

Oxygen microelectrode

The multiwire surface Clark-type oxygen microelectrode (Mehrdracht Oberfläche, Eschweiler, Kiel, Germany) was used for skeletal muscle oxygen pressure (P_tO_2) distribution measurements.[8–10] The electrode consists of eight individual platinum wires, each 15 μm in diameter, embedded in a glass cylinder covered with a cellophane membrane, 12 μm thick. This multiwire microelectrode was calibrated at 37 °C to 0% O_2 (Zero solution, Radiometer, Copenhagen, Denmark) and in distilled water exposed to room air, i.e. to 21% O_2.

One hundred and twenty P_tO_2 values were sampled for each measuring situation over a 4 min period during which the electrode was rotated every 15 s between samplings. As six animals were used in each group, this equals a total number of P_tO_2 values of 720 (120×6) at each assessment time in the respective group. The data were stored for subsequent data analysis and calculation on a hard disc in a PC (OMtech 386), through a 13 bits resolution AD-converter. An equilibration period of at least 5 min was allowed after the electrode had been put in the lucite ring holder before any measurements were started. Hence, measurements of P_tO_2 were made at 10, 30 and 45 min after release of the tourniquet. After the last measurements on the post-ischaemic muscle, an assessment of P_tO_2 in the normally perfused, contralateral muscle was performed, which was designated as the control value for the P_tO_2 in that animal.

Statistics

All data showed non-parametric distribution. Therefore, for statistical analysis the Wilcoxon signed-rank sum test and Mann–Whitney U-test were used in combination with the Friedman and Kruskal–Wallis test. For all statistical evaluation $p<0.05$ was considered significant. The data are presented as histograms or median values (with 25% and 75% percentiles).

Results

After 10 min of reperfusion the median P_tO_2 in group I, exposed to 3 h of ischaemia, was 0.28 kPa, and 0.18 kPa in group II, exposed to 4 h of ischaemia. After 30 min of reperfusion the median P_tO_2 was 0.51 kPa in group I and 0.57 kPa in group II. After 45 min of reperfusion the median P_tO_2 was 0.61 kPa and 0.60 kPa, respectively (Table 1). The median P_tO_2 in the non-ischaemic control muscle was 2.19 kPa and 2.17 kPa in group I and II, respectively (Table 1).

The P_tO_2 histograms in the respective groups are shown in Figs 1 and 2. During reperfusion a similar P_tO_2 pattern was seen in both groups with the sum P_tO_2 distributions shifted to the left, compared to controls. After 10 min of reperfusion the P_tO_2 readings

Table 1. Skeletal muscle tissue oxygen pressure (P_tO_2) in the early reperfusion phase after 3 h (group I) and 4 h (group II) of ischaemia, and in the non-ischaemic control muscle. Values are given as median values (and 25% and 75% percentiles).

Reperfusion time	Group I	Group II
10 min		
P_tO_2 (kPa)	0.28	0.18*
25%	0.15	0.00
75%	0.65	0.47
30 min		
P_tO_2 (kPa)	0.51	0.57**
25%	0.16	0.24
75%	0.86	0.92
45 min		
P_tO_2 (kPa)	0.61	0.60†
25%	0.25	0.27
75%	1.06	0.97
Control		
P_tO_2 (kPa)	2.19‡	2.17†‡
25%	1.52	0.90
75%	2.82	3.31

*$p<0.001$, **$p<0.01$, and †=not statistically significant compared to group I. ‡$p<0.001$ compared to the above states in the same group.

were regularly very low and the distributions showed the majority of the P_tO_2 values close to zero. After 45 min of reperfusion the P_tO_2 distributions had improved, their distributions were less left-shifted, but still subnormal. After 45 min of reperfusion some zero P_tO_2 readings could be found, but their prevalence had diminished.

Discussion

The present study demonstrated severely impaired skeletal muscle oxygenation during the initial 45 min of reperfusion after prolonged ischaemia of both 3 and 4 h duration, assessed by measuring the distributions of P_tO_2 readings from a multi-point surface oxygen electrode. During reperfusion after both 3 and 4 h of ischaemia, a similar left-shifted, post-ischaemic P_tO_2 distribution pattern was found in the histograms with lowered or near zero oxygen pressure values. This suggests that a slow-reflow phenomenon is present during the initial reperfusion with a severely impaired microcirculation and a subnormal tissue oxygenation prevailing long after the restitution of tissue perfusion, despite the pronounced post-ischaemic tissue needs. We propose that this post-ischaemic reperfusion impairment in reoxygenation is caused by a major reduction in local nutritional, microvascular blood flow rate. It is difficult to accept that it should be due mainly to a post-ischaemic reduction in local oxygen consumption, as has been suggested, since that would

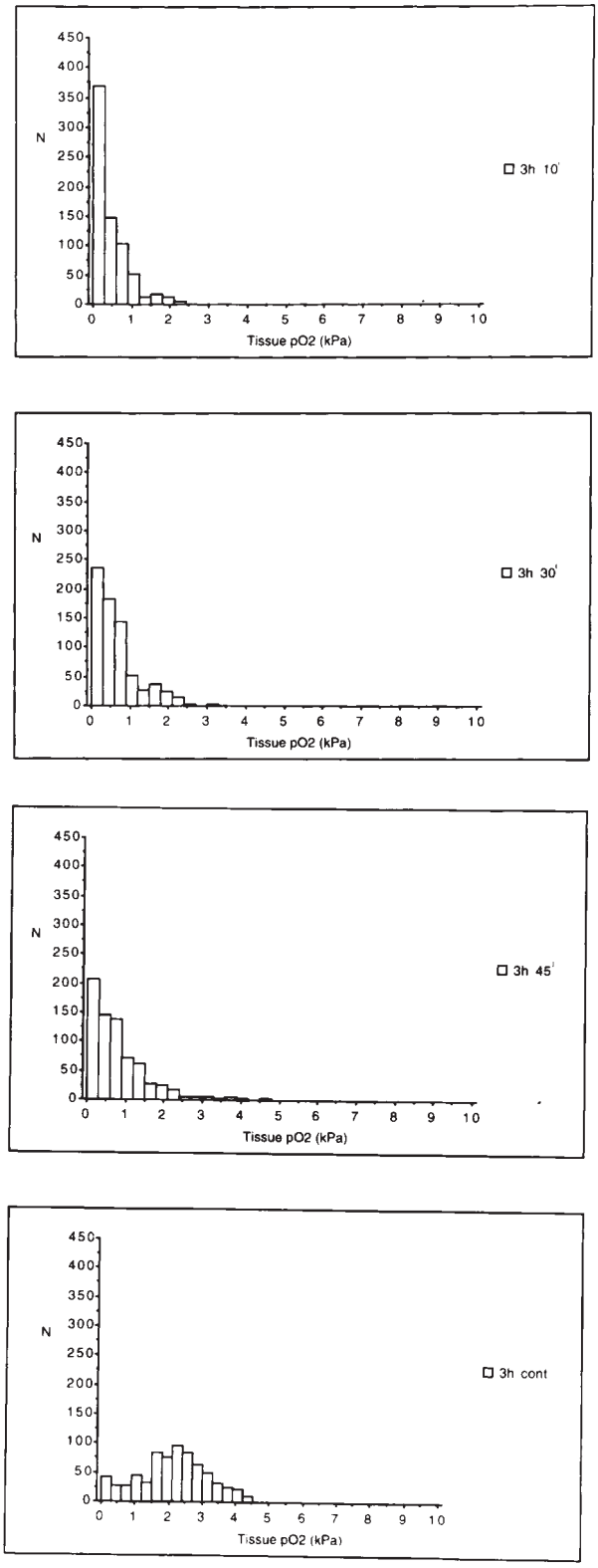

Fig. 1. Skeletal muscle tissue PO_2 distributions (PO_2 classes of 0.3 kPa) after 10 min (10′) ($n=720$), 30 min (30′) ($n=720$) and 45 min (45′) ($n=720$) of reperfusion after 3 h of total ischaemia (group I) and in their normally perfused, contralateral, control muscles (cont) ($n=720$). n=number P_tO_2 values sampled in six animals.

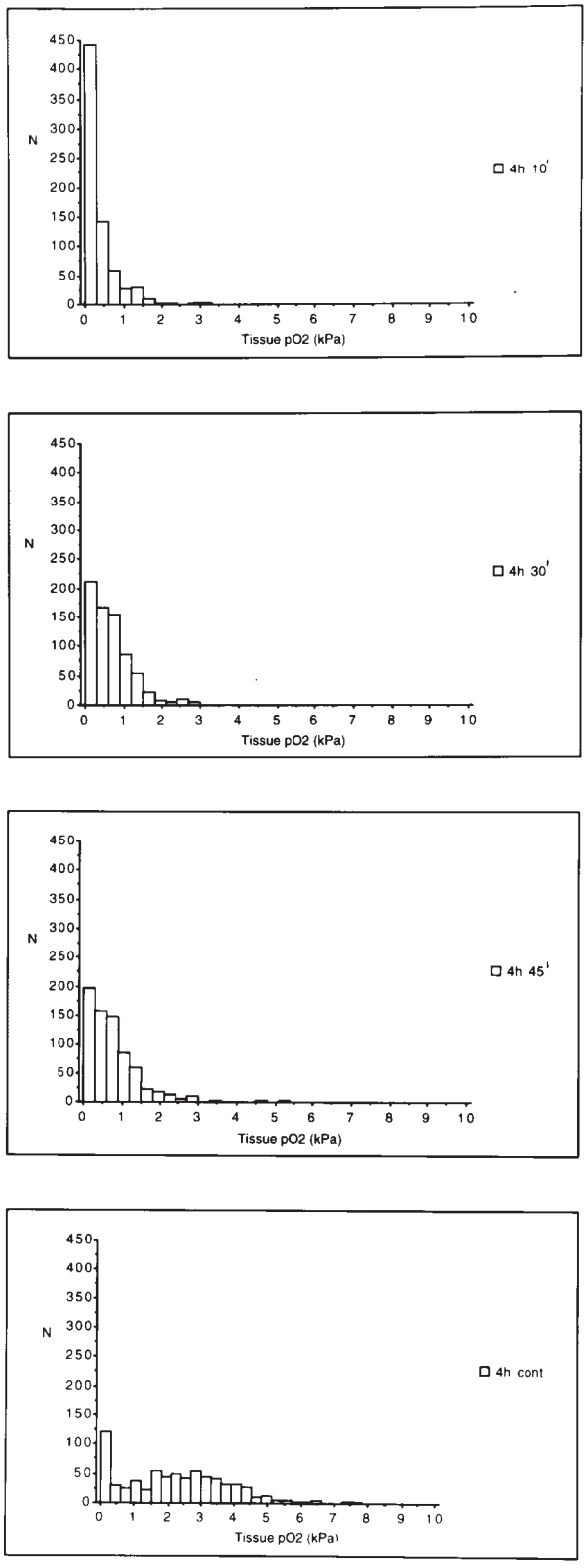

Fig. 2. Skeletal muscle tissue PO_2 distributions (PO_2 classes of 0.3 kPa) after 10 min (10′) ($n=719$), 30 min (30′) ($n=720$) and 45 min (45′) ($n=720$) of reperfusion after 4 h of total ischaemia (group II) and in their normally perfused, contralateral, control muscles (cont) ($n=704$). n = number P_tO_2 values sampled in six animals.

only diminish tissue oxygen extraction and thus give high P_tO_2 readings.[10]

The Clark-type surface multiwire microelectrode has been shown to be a valid and reliable method for measurement of the tissue oxygen pressure[8,9] not only in skeletal muscle,[10,12] but also in other tissues such as the heart, brain and the gut.[13–15] As the oxygen pressure in skeletal muscle is heterogeneously distributed and varies from zero up to arterial levels,[12] single measurements of P_tO_2 are of limited value. Using the surface Clark-type multiwire electrode, a large number of individual oxygen pressure readings from a surface of a tissue volume can be sampled. This makes it possible to construct oxygen pressure histograms, which thus provide a more valid description of the composite oxygen concentration in the tissue volume, as compared to measurements sampled by a one-point microelectrode. The catchment distance of each wire using the Clark-type oxygen electrode is assumed to be approximately 20 μm.[13] Thus, this electrode gives a spatial resolution of one or two capillaries in skeletal muscle.[16] However, this type of surface measurement is open to the criticism that it may not accurately reflect the oxygen tensions within the deep volumes of the tissue. It has been shown that a more severe post-ischaemic or hypoxic damage is seen deep in the muscle belly compared to that in surface layers of the rat tibialis anterior muscle exposed to 4 h of ischaemia.[17] Such integrated volume measurements of P_tO_2 must await other measuring techniques than presently available, possibly using NMR spectroscopic measurements.

The present measurements were started after 10 min of reperfusion for practical reasons. The initial local skeletal muscle blood flow response after 3 h of ischaemia in rats is a slight initial reactive hyperaemia, with a peak some minutes after the start of reperfusion.[6] Thereafter the blood flow rates are reduced to baseline within 10 min of reperfusion.[1] After that time period a pronounced hypoperfusion is seen, indicating the persistence of an impaired reoxygenation, possibly caused by a late post-ischaemic microvascular hindrance to perfusion or a post-ischaemic imbalance between the vasodilatory and vasoconstrictive components within the resistance portions of the microvascular tree, failing to achieve the necessary vasodilation.[1,5] The mechanism behind this type of no-reflow phenomenon in skeletal muscle may include factors such as white cell plugging caused by either loss of white cell corpuscle deformability,[18,19] and/or a post-ischaemically increased expression of tethering and adhesion molecules on the surface of the microvascular endothelium and/or endothelial swelling due

to a post-ischaemic loss of endothelial cell volume control.[20] At the same time post-ischaemic changes in permeability will cause the development of oedema.[21,22] Moreover, impaired blood fluidity in the hypoperfusion state could also play an important role in the pathogenesis of the no- or slow reflow phenomenon following prolonged ischaemia.[4,23]

Using a window preparation to study the tissue oxygenation in the dorsal cutaneous muscle in awake hamsters, it was observed that the muscle mean PO_2, as measured with the Clark-type multiwire electrode, decreased from 2.8 kPa prior to ischaemia down to 1.2 kPa after 15 min of reperfusion following 4 h of total ischaemia.[23] This post-ischaemia reduction is of the same magnitude as presently found. In the above study a decrease in functional capillary density seemed to occur early during the reperfusion phase, causing the observed reduction in muscle oxygenation. The hypoperfusion was still observed 24 h after the ischaemic insult, when only a moderate recovery of P_tO_2 was detected, indicating the time-scale of the post-ischaemic reperfusion impairment.[23]

A major number of the sampled P_tO_2 values registered in this study were found to be close to zero during the first 45 min of reperfusion after both 3 and 4 h of tourniquet ischaemia. This pronounced left-shifted type of P_tO_2 distributions indicate a profound tissue anoxia and that many capillaries would have been non-perfused. However, a moderate number of low P_tO_2 values was also seen in the contralateral, normally perfused, control muscle. Such findings were more frequently found during reperfusion in animals exposed to 4 h of ischaemia than in those exposed to 3 h. Likewise, very low local tissue PO_2 values have also been observed at some tissue sites during reperfusion after hemorrhagic shock in rabbit skeletal muscle.[24] Those findings during reperfusion were interpreted as a result of no-reflow or slow-reflow phenomenon in the hypovolemic period, the causes of which remain to be revealed.

In conclusion, the present observations show that the local skeletal muscle oxygenation is severely impaired in rat skeletal muscle during the initial reperfusion time period after both 3 and 4 h of ischaemia. This indicates that a slow-reflow phenomenon is present during reperfusion after prolonged ischaemia with markedly reduced skeletal muscle tissue reoxygenation, reflecting a post-ischaemic impairment in the nutritional microvascular blood flow regulation.

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